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DEVELOPMENT OF AN LC-ICP-MS METHOD FOR ZINC SPECIATION IN FISH FEEDS

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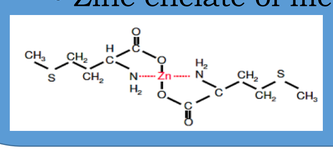
INTRODUCTION

- In analytical chemistry, speciation is considering individually the different chemical species of an element. Information regarding speciation is very important since the biological role of any particular element greatly depends on its chemical form.
- Nowadays, fish feeds are largely plant-based. Among the microminerals essential to fish, zinc is one of the most limited in plant-based diets. In order to meet fish requirement, there is a need to supply zinc in feeds.
- One of the objectives of the project is to study zinc requirement in Atlantic salmon. For that reason, speciation methods using LC-ICP-MS will be developed, aiming to quantify and identify the different zinc chemical forms present in feed ingredients, feeds and salmon tissues.

OUTLINE

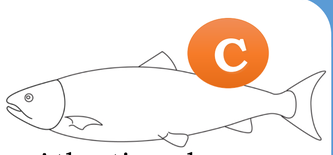
A variety of Zn-feed additives are approved by EU[1], to be used in fish feeds, for instance:

- Zinc acetate
- Zinc oxide
- Zinc sulphate
- Zinc chelate of glycine
- Zinc chelate of methionine



A — Plant ingredients (wheat, pea, corn, …)

B — Plant-based feeds



C — Atlantic salmon (fillet, faeces, …)

SPECIATION:
- Mild extraction
- IEC-ICP-MS
- SEC-ICP-MS

TOTAL CONTENT:
- HNO_3 digestion
- ICP-MS

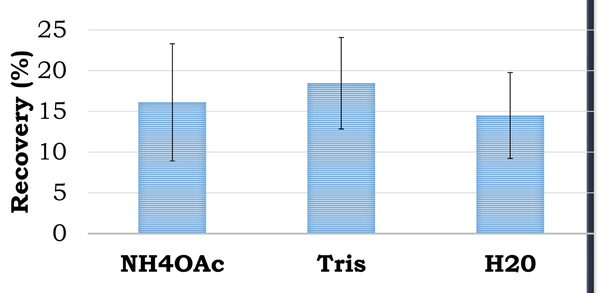
Zn concentrations: Mean (Min.-Max.)	
A	41 (19-51) mg/kg, n=10 (¤)
B	163 (110-400) mg/kg, n=73 (¤)
C	0.36 (0.20-0.60) (w/w) mg/kg, n=40 (¥)

(¤) Data from 2015, Annual feed surveillance programme performed by NIFES on behalf of the Norwegian Food Safety Authority.
(¥) Data from 2006, seafood data at NIFES.no.

PRELIMINARY RESULTS

1 Solubility Tests

Feed samples were mixed with 10 mL of each solution (20 mM of NH_4OAc, pH 7.3; 50 mM Tris buffer, pH 7.5; H_2O) for 1h, using a rotator (30 rpm). Recovery was determined comparing Zn in extract with total Zn feed.

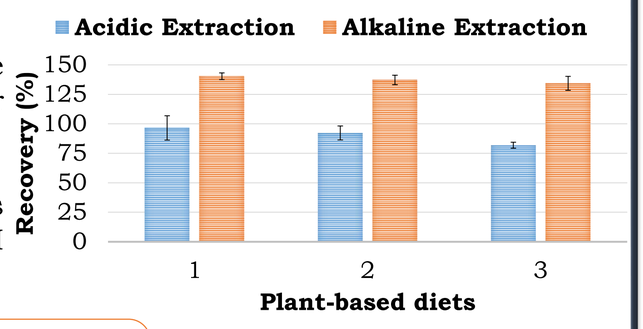


Results presented as the mean ± SD, n=3.

2 Extraction Methods

Acidic Extraction: Feed samples were mixed with 5 mL of 0.1 M HNO_3 for 1h, using a water bath, at 90°C.

Alkaline Extraction: Feed samples were mixed with 5 mL of 0.1M NaOH for 20h, using an elliptic table.



3 Speciation

- Zn acetate
- Zn oxide
- Zn sulphate

SAX: PRP-X100, Hamilton

Chromatographic conditions:

50 mM $(NH_4)_2CO_3$ + 3% MeOH (pH 7), isocratic gradient

- All compounds are retained (±9 min.), showing similar chromatographic profile

SCX: Ionosphere 5C, Agilent

Chromatographic conditions:

(A) 0.5 mM Pyr + 3% MeOH (pH 3)

(B) 10 mM Pyr + 3% MeOH (pH 3)

0-15 min., 50% A + 50% B

15-30 min., 100% B

30-35 min., 50% A + 50% B

- Compounds are not retained

CONCLUSIONS & NEXT STEPS

1
- Based on solubility tests, it was seen that slightly better recovery is obtained when using Tris buffer (pH 7.5).
- Lower Zn recovery may be related to Zn complexing with chemical structures that are not soluble.
- Next experiments will be done using longer time of extraction and protease and phytase will be added to study if Zn is binding to phytic acid and peptides.

2
- Acidic extraction gave almost quantitative recovery in the feeds analysed (mean recoveries in the range of 81-96%).
- Alkaline extraction gave recoveries in the range of 134-140%. The high recoveries may be related to matrix effects during analysis. The analysis will be repeated with standard addition calibration to correct for these effects.

3
- Based on the preliminary results, further experiments will be performed using anion-exchange chromatography (PRP-X100).
- Different chromatographic conditions will be tested and Zn organic bound compounds will be evaluated.
- Possible difficulties with stability of Zn compounds in solution it is an issue to keep in mind.
- SEC–ICP–MS will be tested with Zn organic compounds including feed additives and natural occurred compounds.

REFERENCES

[1] Reg (EC) No 1831/2003. European Union Register of Feed Additives. Edition 224. Appendixes 3e, 4 – 03.02.2016

ACKNOWLEDGEMENTS

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